



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/DK93/00152 <b>(22) International Filing Date:</b> 5 May 1993 (05.05.93) <b>(30) Priority data:</b> 0601/92 8 May 1992 (08.05.92) DK <b>(71) Applicant (for all designated States except US):</b> NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> NIELSEN, Per, Munk [DK/DK]; Rytterstien 29A, DK-3400 Hillerød (DK). PETERSEN, Bent, Riber [DK/DK]; Strandvænget 1, DK-2100 København Ø (DK). BUDOLFSEN, Gitte [DK/DK]; Lyøvej 3, 3.tv, DK-2000 Frederiksberg (DK).		<b>(74) Common Representative:</b> NOVO NORDISK A/S; Patent Department, Novo Allé, DK-2880 Bagsværd (DK). <b>(81) Designated States:</b> AU, CA, JP, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> METHOD FOR PRODUCTION OF A MILK LIKE PRODUCT, THE MILK LIKE PRODUCT, AND USE OF THE MILK LIKE PRODUCT  <b>(57) Abstract</b>  The method for production of a milk like product is characterized by the fact that transglutaminase is added to a liquid containing milk proteins, that the liquid contains Ca <sup>++</sup> , if required, in an amount sufficient for the reaction catalyzed by the transglutaminase, and that the pH of the liquid, if required, is adjusted to a value of between 5.5 and 7.5. Hereby a milk like product which is physically more stable than the hitherto known milk like products is obtained. By further addition of an enzyme with milk clotting activity a milk like product with a mouth feeling like a fat emulsion and usable as a fat substitute can be produced.		

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## METHOD FOR PRODUCTION OF A MILK LIKE PRODUCT, THE MILK LIKE PRODUCT, AND USE OF THE MILK LIKE PRODUCT

The present invention concerns a method for production of a milk like product, the milk like product, and use of the milk like product.

5 In this specification with claims a milk like product is to be understood as comprising a product produced with a liquid containing milk proteins from an animal source as a starting material. Examples of liquids containing milk proteins from an animal source are cow milk, buffalo milk, goat milk, sheep milk, and animal milk fortified with casein or whey proteins.

10 It is known that milk is physically unstable to protease attack, forming a coagulum after exposure to proteases, e.g. rennet. This is a desirable property in regard to cheese production. In other cases, however, this property is undesirable, especially in relation to long term storage stability of UHT milk. Even if the microorganisms are killed during the UHT treatment, thermostable proteases  
15 produced by certain microorganisms may not be completely inactivated during the UHT treatment, and in such cases the UHT milk is physically unstable during long term storage.

Thus, it is the purpose of the invention to provide a method for provision of a milk like product, which is physically more stable than the hitherto  
20 known milk like products.

Surprisingly, according to the invention it has been found that the purpose of the invention can be fulfilled, if transglutaminase is added to a liquid containing milk proteins and  $\text{Ca}^{++}$ , if required. As an explanatory remark it can be added that the skilled worker in the art will be aware that some transglutaminases  
25 require  $\text{Ca}^{++}$  in order to exhibit enzymatic activity, and others do not.

Thus, the method for production of a milk like product according to the invention is characterized by the fact that transglutaminase is added to a liquid containing milk proteins, that the liquid contains  $\text{Ca}^{++}$ , if required, in an amount sufficient for the reaction catalyzed by the transglutaminase, and that the pH of the  
30 liquid, if required, is adjusted to a value of between 5.5 and 7.5.

Thus, it has been found that the milk like product produced by means of the method according to the invention is extremely stable in comparison to the prior art milk like products. Documentation will be presented later in this specification. Especially it has been found that the milk like product according to the invention, if UHT treated, exhibits a satisfactory long term physical storage stability.

If according to the invention cow milk is used as the liquid containing milk proteins it has been found that the milk like product according to the invention exhibits an excellent physical stability to protease attack.

The amount of transglutaminase to be added depends on the reaction time. If the reaction time is long, e.g. 2 hours, very little transglutaminase is necessary in order to generate the stability.

From Japanese unexamined patent application no. JP-A-3160957 it appears that a material containing novel modified milk protein can be obtained by letting transglutaminase act on a liquid product containing milk protein, and by subsequent heating and drying. Also, it appears that the object of the Japanese invention is the provision of a product, which is able to form a gel on heating. However, it does not appear from the Japanese citation that a new production method for physically stable UHT milk can be obtained if the heating and drying is omitted, as indicated in this specification, and a skilled worker in the art familiar with the Japanese citation would not find the slightest incentive to omit the heating and the drying.

A preferred embodiment of the method according to the invention is characterized by the fact that the transglutaminase is of human, of bovine or of microbial origin. In this manner the method according to the invention can be carried out in an economically sound manner.

A preferred embodiment of the method according to the invention is characterized by the fact that the transglutaminase is activated factor XIII. In this manner the method according to the invention can be carried out in an economically sound manner.

A preferred embodiment of the method according to the invention is characterized by the fact that the liquid containing milk proteins is milk from

domestic animals, preferably from cows. This is a cheap and easily available starting material.

A preferred embodiment of the method according to the invention is characterized by the fact that an enzyme with milk clotting activity is added to the liquid containing milk proteins. This embodiment, which is illustrated in Example 4, comprises only the cases, in which a milk like product is produced. If for instance the enzyme with milk clotting activity is added ample time before the addition of transglutaminase, a cheese like product is produced, and thus, this case is outside the scope of the invention. Surprisingly, it has been found that this embodiment inside the scope of the invention gives rise to a special milk like product, i.e. a milk like product with microparticulated protein, aggregated by means of the enzyme with milk clotting activity. This milk like product exhibits a mouth feeling like a fat emulsion. Similar milk like products with microparticulated protein is described in WO 91/17665 and EP 468,560. However, the product described in WO 91/17665 often gets a burned taste due to the necessary heat treatment, and the method described in EP 468,560 involves a time consuming fermentation process, which is not performed during the method according to the invention.

A preferred embodiment of the method according to the invention is characterized by the fact that an enzyme with milk clotting activity is added to a casein solution. In this manner a lactose free milk like product is produced. It can be used in all cases, where the presence of lactose should be avoided.

A preferred embodiment of the method according to the invention is characterized by the fact that the enzyme with milk clotting activity is a cheese rennet enzyme. This enzyme is commercially easily available.

The invention also comprises a milk like product, which is characterized by the fact that it is produced by means of the method according to the invention.

Also, the invention comprises a use of the milk like product according to the invention as a fat replacer in foods. Typical examples of foods, in which the milk like product according to the invention can be incorporated as a fat replacer, are ice cream, toppings, frozen desserts, dressings, mayonnaises and low fat spreads. As a consequence of this use it is possible to produce foods which have

a lower calorie content, and which still possess organoleptic properties similar to the organoleptic properties of foods with a normal fat content.

#### EXAMPLE 1

As a starting material 200 ml of whole cow milk was used.  $\text{Ca}^{++}$  as  $\text{CaCl}_2$  was added to a concentration of 5 mM  $\text{Ca}^{++}$ . The pH was adjusted to 6.7, and the temperature was adjusted to 32°C. Transglutaminase was added as 30 mg of activated factor XIII. Also, a control experiment without transglutaminase was carried out. This mixture was left alone for 30 minutes. Subsequently 0.12 g of Rennilase® 14XL rennet was added to the mixture. A heavy clotting took place in the control, but no clotting took place in the milk like product according to the invention.

#### EXAMPLES 2 AND 3

Example 1 was repeated, except for the fact that the addition of factor XIII and the rennet was performed simultaneously (Example 2), and except for the fact that the additions of factor XIII and the rennet were reversed, whereby the addition of factor XIII was carried out immediately after the addition of rennet (Example 3). Also in these cases a heavy clotting took place in the control, but no clotting took place in the milk like product according to the invention.

#### EXAMPLE 4

As a starting material 100 ml of skim milk of 32°C was used, and  $\text{Ca}^{++}$  was added to a total concentration of 5 mM. The pH-value was adjusted to 6.7. Rennilase® (an enzyme with milk clotting activity) was added in a concentration of 6.5 RU/ml, the RU unit being defined in IB 67/3-e (a Novo Nordisk product information sheet, available upon request from Novo Nordisk A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark. After 5 seconds activated factor XIII was added in a concentration of 0.4% w/w, based on the protein content. A milk like product with microparticulated protein and with a mouth feeling like a fat emulsion was produced.

**CLAIMS**

1. Method for production of a milk like product, wherein transglutaminase is added to a liquid containing milk proteins, wherein the liquid contains  $\text{Ca}^{++}$ , if required, in an amount sufficient for the reaction catalyzed by the transglutaminase,  
5 and wherein the pH of the liquid, if required, is adjusted to a value of between 5.5 and 7.5.
2. Method according to Claim 1, wherein the transglutaminase is of human, of bovine or of microbial origin.
3. Method according to Claim 1, wherein the transglutaminase is activated  
10 factor XIII.
4. Method according to Claims 1 - 3, wherein the liquid containing milk proteins is milk from domestic animals, preferably from cows.
5. Method according to Claims 1 - 4, wherein an enzyme with milk clotting activity is added to the liquid containing milk proteins.
- 15 6. Method according to Claim 5, wherein the liquid containing milk proteins is a casein solution.
7. Method according to Claim 5 or 6, wherein the enzyme with milk clotting activity is a cheese rennet enzyme.
8. Milk like product, characterized by the fact that it is produced by means  
20 of the method according to Claims 1 - 7.
9. Use of the milk like product according to Claim 8 as a fat replacer in foods.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 93/00152

## A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A23C 11/00, A23C 9/12, A23J 3/08, A23L 1/305  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A23C, A23J, A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CLAIMS/US PATENTS, CA, BIOSIS, AGRICOLA, CAB, FSTA, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Service, File 351, WPIL, Dialog accession no. 00874421, WPI accession no. 91-248437/34, (AJIN): "Food material contg. denatured milk proteins - obtd. by treating liq. contg. milk proteins with trans-glutaminase, heating and drying.", & JP,A,3160957, publ. 910710 --	1-9
A	Dialog Information Service, File 50, CAB, Dialog accession no. 1258838, CAB accession no. OD054-03006, Rao, D.S.: "Enzymatic modification of milk proteins during processing - a possible role for native milk proteinase.", Indian Dairyman (1991) 43 (11) p. 514-517 --	1-9

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Service, File 351, WPIL, Dialog accession no. 008465483, WPI accession no. 90-352483/47, (AJIN): "Minced meat and fish paste - contains soybean protein and/or milk protein and transglutaminase.", & JP,A,2255060, publ. 901015 --	1-9
A	DE, C, 181918 (EMMERICH, R.), 8 March 1905 (08.03.05), claim 1 -- -----	1-9

### Information on patent family members

International application No.

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